PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/011183	07.10.2004	10.10.2003

International Patent Classification (IPC) or both national classification and IPC
C12P7/42, C12P13/00, C12N1/00

Applicant
DEGUSSA AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Döpfer, K-P

Telephone No. +49 89 2399-8547

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/011183

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material:

      ☐ in written format

      ☐ in computer readable form

   c. time of filing/furnishing:

      ☐ contained in the international application as filed.

      ☐ filed together with the international application in computer readable form.

      ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-8,10,11
	No:	Claims	9
Inventive step (IS)	Yes:	Claims	10,11
	No:	Claims	1-9
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Re Item I

Basis of the report

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: OSPRIAN INGRID ET AL: "Biocatalytic hydrolysis of cyanohydrins: An efficient approach to enantiopure alpha-hydroxy carboxylic acids." JOURNAL OF MOLECULAR CATALYSIS B ENZYMATIC, vol. 24-25, 1 October 2003 (2003-10-01), pages 89-98, XP002314143 ISSN: 1381-1177

D2: GRIENGL H ET AL: "The synthesis of chiral cyanohydrins by oxynitrilases" TRENDS IN BIOTECHNOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 18, no. 6, June 2000 (2000-06), pages 252-256, XP004203650 ISSN: 0167-7799

D3: EP-A-0 632 130 (CHEMIE LINZ GMBH) 4 January 1995 (1995-01-04)

D4: EP-A-0 711 836 (NITTO CHEMICAL INDUSTRY CO LTD) 15 May 1996 (1996-05-15)

D5: EP-A-0 446 826 (KERNFORSCHUNGSANLAGE JUELICH) 18 September 1991 (1991-09-18)

2. *Novelty and Inventive Step (Article 33(2)(3) PCT)*

2.1 *Subject-matter of the present application*

The present application addresses (i) an enzymatic method for preparing enantiomer-enriched α-hydroxycarboxylic acids or their respective amides from aldehydes or ketones as starting compounds with oxynitrilase (EC 4.1.2.10 or 4.1.2.11) and nitrilase (EC 3.5.5.1) or nitrile hydratase (EC 4.2.1.84); (ii) an enzyme system comprising aldehydes or ketones, a cyanide donor, an oxynitrilase, and a nitrilase or a nitrile hydratase; (iii) a whole cell catalyst exhibiting a cloned gene for an oxynitrilase and a nitrilase or a nitrile hydratase.

2.2 *Novelty*

The prior art does not disclose a method for preparing α-hydroxycarboxylic acids or the respective amides using a two-enzyme system comprising oxynitrilase in presence of nitrilase or nitrile hydratase. Neither is a genetically engineered microorganism known which exhibits both oxynitrilase and nitrilase or nitrile hydratase activity. The subject-matter of present claims 1-8, 10 and 11 is accordingly novel over the prior art.

Claim being directed to a "*system comprising ...*" does not say that all components must be in one spatial entity. D1 discloses a two-step synthesis of α-hydroxycarboxylic acids comprising all components of claim 9, although not in one apparatus. The term "system" interpreted in it's broadest sense encompasses also a plurality of apparatuses (located e.g. in a laboratory) comprising the components required by the claim. Thus, claim 9 is not novel in view of D1.

2.3 *Inventive Step*

D1 is considered as representing the closest prior art. It discloses the two-step synthesis of chiral α-hydroxycarboxylic acids from aldehydes or ketones using in the first step oxynitrilase and in a second step nitrilase. The problem underlying the present application can be seen as to provide an alternative method for the enzymatic preparation of α-hydroxycarboxylic acids, e.g. an one-step procedure. The solution is the method of present claim 1, i.e. the synthesis of α-hydroxycarboxylic acids or amides via an enzymatic method for preparing enantiomer-enriched α-hydroxycarboxylic acids or their respective amides from aldehydes or ketones as starting compounds with oxynitrilase (EC 4.1.2.10 or 4.1.2.11) in presence of nitrilase (EC 3.5.5.1) or nitrile hydratase (EC 4.2.1.84).

D1 discusses the problem of the enzymatic synthesis of cyanohydrins with oxynitrilase concerning the reversibility of the cyanohydrin formation which would require a high velocity of the first reaction step, i.e. the resting cells of *Rhodococcus* exhibiting a highly active nitrile hydratase/amidase system must be freshly prepared in order to shift the equilibrium of the cyanohydrin formation to the product. It is obvious to the skilled person that the immediate reaction of the intermediate product cyanohydrin with a suitable enzyme (or cell exhibiting that required activity) would solve that problem. Documents D2 to D5 provide the skilled person with sufficient information concerning either the enzymes or the sources where the enzymes can be obtained from and which are suitable to be

used in such a procedure. The skilled person would have taken D1 alone or in combination with either D2 to D5 into consideration in order to come to the proposed solution of Claims 1 to 8, the subject-matter of claims 1-9 in it's broadest sense does not involve an inventive step (see also Item VII of this written opinion).

3. *Industrial applicability (Article 33(4) PCT*

The subject-matter of present claims 1-11 appear to comply with the requirements of industrial applicability as stipulated in Article 33(4) PCT.

Re Item VII
Certain defects in the international application

1. The applicant states at page 4, lines 20 et sequence. of the application that the incompatibility of the reaction conditions of both the cyanohydrin formation and the nitrile saponification (better called hydrolysis) has to be taken into consideration when designing a "one-step" method. The scope of claim 1 encompasses any variant where both the oxynitrilase and the nitrilase/nitrile hydratase enzymes are present in the reaction mixture. The application is however silent how the incompatibility can be overcome. The suggested cloning of both enzyme in a host cell is also not disclosed in such a manner that the measures are shown how to get a system which allows both reactions to take place in a single environment. The application is therefore regarded as not to meet the requirements of Article 5 PCT in respect to sufficient disclosure. It is obvious that the skilled person could perform the genetic engineering of a suitable host cell in order to get the expression of both enzymes. But the crucial steps to make them operating in a defined reaction environment which would lead to the successful reaction sequence carbonyl compound - cyanohydrin - α-hydroxycarboxylic acid/amide are neither disclosed not even contemplated or discussed.

2. The use of EP and WO application numbers (pages 7, 11 and 12) is not in accordance with the requirements of Rule 5.1.(a)(ii) PCT because documents not yet published do not belong to the prior art as stipulated in Rule 64 PCT.